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Applicant : Kambe et al.

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BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

I, Terry M. Bricker, Ph.D., hereby declare as follows:

1. I am presently the Mooreland Family Professor of Basic Sciences, Department of Biological Sciences and an adjunct Professor of Chemistry at Louisiana State University, Baton Rouge, Louisiana.
2. I received my Ph.D. degree in 1981 in Botany from Miami University.
3. I have been on the faculty at the Louisiana University since 1987. I was promoted to Associate Professor with tenure in 1990 and to full Professor in 1994. A copy of my Resume is attached.
4. I have been Visiting Professor at the University of Illinois and Michigan State University. I am the author or co-author of many scientific articles, conference proceedings and review papers. I have served on the editorial board of Plant Physiology and the Annual Reviews of Plant Physiology and Plant Molecular Biology.
5. I have no financial interest in NanoGram Corporation or in the present patent application.

6. I have extensive experience in separation technology applied to biological nanoparticles, in particular, proteins and DNA. I and coworkers in my laboratory continuously use several forms of chromatography in the separation and purification of proteins. I have used chromatography and protein purification techniques throughout my career.

7. I have read carefully U.S. Patent 5,442,254 to Jaskie (the Jaskie patent). I have evaluated the description of particle separation in the Jaskie patent based on my extensive expertise in separation technology generally.

8. The isolation method of the quantum particles describe in the Jaskie patent at column 7, lines 28-40 relies on the use of capillary action to separate particles of different diameters. Specifically, particles with a diameter range of about 10 to 100 angstroms are suspended in a liquid, and the liquid is allowed to move up a cloth by capillary action. The authors argue that the distance which various particles migrate up the cloth is directly proportional to their size. The authors further suggest that at any given height up the cloth all of the particles will be the same size. Thus, the authors are describing a chromatographic system which they allege will differentially fractionate the particles based on size.

9. The separation techniques described in the Jaskie patent will not separate different size classes of quantum particles. First, a mixture of different sized particles is continuously loaded onto the cloth. Even assuming for argument that the different sized particles migrate at different speeds, additional particles are continuously loaded behind the migrating edge of initially loaded particles. Thus, the particles are continuously remixed with particles of other sizes as additional particles are loaded onto the cloth. This remixing occurs for every size class of particles. Significantly, the technique will not work because no cloth is known with the necessary properties to differentially interact with different sized particles.

Traditional thin layer chromatography is based on differential solubility constants for the chemicals being separated. Due to different solubility constants, solutes migrate at different rates as the solvent is taken up by capillary action.

10. Any chromatographic separation approach relies on 1) the properties of the liquid that the particles are suspended in, 2) the surface characteristics of the cloth, 3) the surface properties of the quantum particles, and 4) the size of the quantum particles. The patentees provide no direct information on the first three of these categories. Based on the discussion in the previous paragraph in column 7 of the patent, perhaps one can assume that the solvent is water. However, the surface properties of the cloth used in the described separation is critical. Separation in any chromatographic system is dependent on differential partitioning of the solutes, i.e., the quantum particles, between a mobile phase, the water, and a stationary phase, the cloth. However, no such cloth exists. For separation of biological macromolecules, such separation by size is the purview of gel filtration chromatography. Even in gel filtration fractionation systems, the relatively small differential partitioning coefficients observed prevent true high resolution separations. In this particular instance, one must necessarily obtain high degrees of dimensional resolution of the quantum particles to achieve wavelength selectivity. The required dimensional resolution cannot be achieved by the methods presented in column 7, lines 28-40 of the Jaskie patent.


11. The addition of an electric field would not overcome these problems. First, the authors do not make any claim that the surface charge on the particles is directly proportional to the size. The authors do not describe the means of attaching the electric current or even if the current is applied axially or perpendicular to the capillary flow. Critically, the authors do not describe the properties of the cloth even though the properties of the cloth would critically effect the separation in

the presence of the electric field. While gel electrophoresis is used to separate biological macromolecules using electric fields, these separation are performed in polymer gels, not cloth, that have been developed for the specific purpose of separating biological macromolecules. The gel acts as a sieve, allowing the fractionation of the biological macromolecules by size, surface charge and steric properties. No cloth exists with the required sieving properties. Even the protocols effective to separate different types of biological macromolecules, such as water soluble proteins, membrane proteins and nucleic acid fragments, are significantly different from each other. Inorganic particles are very different with respect to chemical properties and chemical structure from biological macromolecules. The Jaskie patent provides no information that guides anyone trying to adapt these biochemical methods to the separation of quantum particles.

Thus, a person familiar with the separation technologies could not separate Jaskie's quantum particles based on information provided in the Jaskie patent.

12. I declare that all statements made herein that are of my own knowledge are true and that all statements that are made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 10/8/00

By: 
Terry M. Bricker, Ph.D.



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RESUME

Dr. Terry M. Bricker
Position: Professor

EDUCATION:

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Miami University Univ. Missouri	Ph.D. Postdoctoral Work	1981 1981-1985	Botany Biological Sciences

RESEARCH AND PROFESSIONAL EXPERIENCE:

National Science Foundation, IGERT Pre-proposal Panel, 9/00.
Interim Chairperson, Department of Biological Sciences, 6/99-8/00.
Moreland Family Professor of Basic Sciences, Department of Biological Sciences, 4/99-Present
Invited Speaker, Gordon Research Conference Photosynthesis-Biochemical Aspects, 6/99.
Editorial Committee, Annual Reviews of Plant Physiology and Plant Molecular Biology - Vol. 52, 10/98.
Adjunct Professor, Department of Chemistry, Louisiana State University, 3/98-Present.
Professor, Department of Biological Sciences, Louisiana State University, 7/97-Present.
Professor and Chairman, Department of Microbiology, Louisiana State University, 7/95-6/97.
National Science Foundation, Research Training Grant site visit team to Penn State University, 6/96
National Science Foundation, Research Training Grant Advisory Panel, 4/96
Discussion Leader, Gordon Research Conference, "Photosynthesis - Biochemical Aspects", 8/96
Visiting Professor, Department of Plant Biology, University of Illinois 1/95-6/95.
Professor, Department of Plant Biology, Louisiana State University, 8/94-7/95.
Visiting Faculty, Plant Biochemistry Intensive Summer Course, MSU-DOE Plant Research Laboratory, Michigan State University, 6/94.
Associate Professor, Department of Botany, Louisiana State University, 8/90-7/94.
Invited Speaker, Gordon Research Conference, "Photosynthesis - Biochemical Aspects", 8/93.
Director, Protein Analysis Center, College of Basic Sciences, 7/90-8/92.

Dr. Terry M. Bricker

Page Two

Graduate Coordinator, Department of Botany, Louisiana State University, 9/89-8/92.

Department of Energy, *Ad hoc* Advisory Panel Basic Energy Biosciences, 1991.
National Science Foundation Molecular Biochemistry Program Advisory Panel, 10/90-4/94.

Assistant Professor, Department of Botany, Louisiana State University, 8/87-8/90.
Editorial Board, *Plant Physiology*, 1/90-7/92.

Assistant Professor, Department of Chemistry, University of Southern Mississippi, 1/85-8/87.

Publications (last five years, only)

56. Rosenberg, C., Christian, J., Bricker, T. M. and C. Putnam-Evans, "Site-Directed Mutagenesis of Glutamate Residues in the Large Extrinsic Loop of the Photosystem II Protein CP 43 Affects PS II Assembly." To appear *Biochemistry*.
55. Bricker, T.M. and Frankel, L.K. "The Role of Carboxylic Acid Residues on the Manganese-Stabilizing Protein in its Binding to Photosystem II." To appear *Biochemistry*.
54. Wu, J., Masri, N., Lee, W., Frankel, L.K. and T.M. Bricker, "Directed Random Mutagenesis in the Large Extrinsic Loop of the CP 47 Protein of Photosystem II." *Plant Molecular Biology* 39,381-386 (1999).
53. Knoepfle, N., Bricker, T.M., and Putnam-Evans, C., "Site-Directed Mutagenesis of the Basic Residues ³⁰⁵R and ³⁴²R in the CP 43 Protein of Photosystem II Affects Oxygen-Evolving Activity in *Synechocystis* 6803." *Biochemistry* 38,1582-1588 (1999).
52. Bricker, T.M., Morvant, J., Masri, N., Sutton, H. and Frankel, L.K., "Isolation of an Oxygen-Evolving Photosystem II Preparation from *Synechocystis* 6803 using a Histidine-Tagged Mutant of CP 47." *Biochimica et Biophysica Acta* 1409, 50-57 (1998).
51. Zubrzycki, I.Z., Frankel, L.K., Russo, P.S. and T.M. Bricker, "Hydrodynamic Studies on the Extrinsic "33 kDa" Protein of Photosystem II." *Biochemistry* 37,13553-13558 (1998).
50. Ghanotakis, D., Tsiotisz, S. and T.M. Bricker, "Polypeptides of Photosystem II: Structure and Function." In: *Plant Photobiology: Photosynthesis and Photomorphogenesis* (Singhal, G.S., Renger, G., Sopory S.K. Irrgang, K.-D. and Govindjee, eds.) pp. 264-291, Narosa Publishing House, New Delhi, India (1998).
49. Bricker, T.M. and L.K. Frankel, "Structure and Function of the 33 kDa Extrinsic Protein of Photosystem II." *Photosynthesis Research* 56:157-173 (1998).

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47. Qian, M., Al-Khalidi, S., Putnam-Evans, C., Bricker, T.M. and R.L. Burnap, "Photoassembly of the Photosystem II (Mn)₄ Cluster in Site-Directed Mutants Impaired in the Binding of the Manganese-Stabilizing Protein." *Biochemistry* 36,15244-15252 (1997).
46. Putnam-Evans, C. and T.M. Bricker, "Site-Directed Mutagenesis of the Basic Residue ³²¹R to ³²¹G in the CP 47 Protein of Photosystem II Alters the Chloride Requirement for Growth and Oxygen-Evolving Activity in *Synechocystis* 6803." *Plant Molecular Biology* 34:455-463 (1997).
45. Putnam-Evans, C., Wu, J., and T.M. Bricker, "Site-Directed Mutagenesis of the CP 47 Protein of Photosystem II: Alteration of Conserved Charged Residues within Lethal Deletions in the Large Extrinsic Loop of CP 47", *Plant Molecular Biology* 32:1191-1195 (1996).
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41. Leuschner, C. and T.M. Bricker, "Interaction of the 33 kDa Extrinsic Protein with Photosystem II: Rebinding of the 33 kDa Extrinsic Protein to Photosystem II Membranes which Contain Four, Two, or Zero 40. Manganese per Photosystem II Reaction Center." *Biochemistry* 35,4551-4557 (1996).
40. Frankel, L.K. and T. M. Bricker, "Identification of Domains on the 33 kDa Extrinsic Protein which are Shielded from NHS-Biotinylation by Intrinsic Photosystem II Components", *Biochemistry* 34,7492-7497 (1995).